

TITLE OF THE INVENTION

4"-KETO AND -4"-AMINO-4"-DEOXY AVERMECTIN
COMPOUNDS AND SUBSTITUTED AMINO DERIVATIVES THEREOF

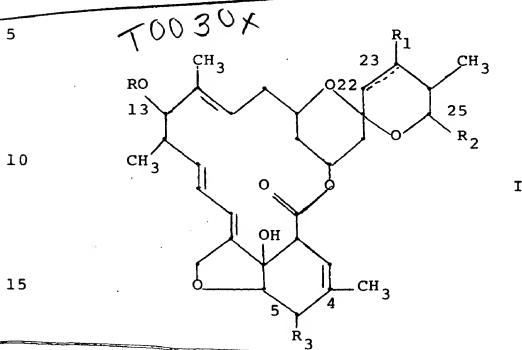
BACKGROUND OF THE INVENTION

P The term avermectin (previously referred to as C-076) is used to describe a series of compounds isolated from the fermentation broth of an avermectin producing strain of Streptomyces avermitilis and derivatives thereof. The morphological characteristics of the culture are completely described in U.S. Patent No. 4,310,519. The avermectin compounds are a series of macrolides, each of which is substituted thereon at the 13-position with a 4-(α -L-oleandrosyl)- α -L-oleandrose group. The avermectin compounds and the instant derivatives thereof have a very high degree of anthelmintic and anti-parasitic activity.

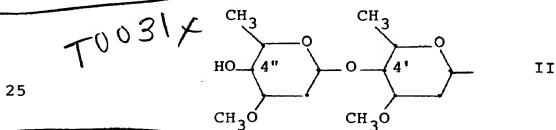
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The avermectin series of compounds isolated from the fermentation broth have the following structure:



PS wherein R is the 4'-(α -1-oleandrosyl)- α -1-oleandrose
20 group of the structure: 60



PS and wherein the broken line indicates a single or a
double bond; R₁ is hydroxy and is present only when
30 said broken line indicates a single bond;

P R₂ is iso-propyl or sec-butyl; and
L R₃ is methoxy or hydroxy.

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There are eight different avermectin natural product compounds and they are given the designations Ala, Alb, A2a, A2b, Bla, Blb, and B2a based upon the structure of the individual compounds.

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In the foregoing structural formula, the individual avermectin compounds are as set forth below. (The R group is 4'(α -L-oleandrosyl)- α -L-oleandrose):

40 (30)

10	Ala	TOO 40 X Double Bond	<u>sec</u> -butyl	-OCH ₃
	Alb	Double Bond	<u>iso</u> -propyl	-OCH ₃
	A2a	-OH	<u>sec</u> -butyl	-OCH ₃
	A2B	-OH	<u>iso</u> -propyl	-OCH ₃
	Bla	Double Bond	<u>sec</u> -butyl	-OH
15	Blb	Double Bond	<u>iso</u> -propyl	-OH
	B2a	-OH	<u>sec</u> -butyl	-OH
	B2b	-OH	<u>iso</u> -propyl	-OH

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- The avermectin compounds are generally isolated as mixtures of a and b components. Such compounds differ only in the nature of the R₂ substituent and the minor structural differences have been found to have very little effect on the isolation procedures, chemical reactivity and biological activity of such compounds.

The end hydroxy group of the disaccharide substituent at the 13-position is situated at what is referred to as the 4"-position. The reactions and substitutions at the 4"-position is the subject matter of this invention.

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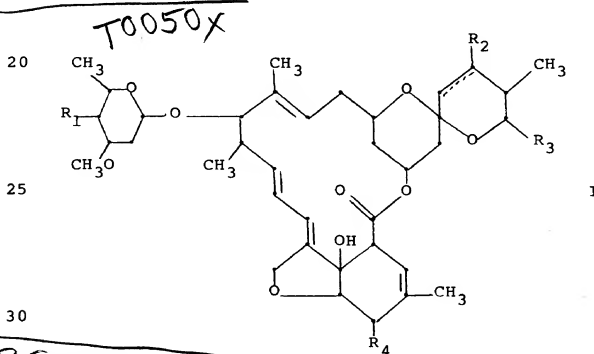
SUMMARY OF THE INVENTION

P The instant invention is concerned with certain derivatives of avermectin compounds wherein the 4"-hydroxy group is oxidized to ketone and replaced by an amino or substituted amino group. Thus it is an object of the instant invention to describe such 4"-substituted avermectin compounds. A further object is to describe processes for the preparation of such compounds. A still further object is to describe the uses of such compounds as anti-parasitic agents and anti-bacterial agents. Still further objects will become apparent from a reading of the following description.

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15 DESCRIPTION OF THE INVENTION

P The compounds of the instant invention have the following structural formula.



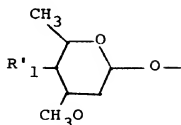
PS

wherein

R_1 is $=O$, $\frac{1}{50}$, $\frac{1}{m}NR_5R_6$ or

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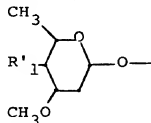
- wherein $R'1$ is $\equiv O$, or $-NR5R6$;
 $R5$ and $R6$ are independently hydrogen,
loweralkyl, loweralkanoyl, substituted
10 benzenesulfonyl wherein the substituent is halogen
or loweralkyl sulfonyl;
 $R2$ is hydrogen or hydroxy,
 $R3$ is iso-propyl or sec-butyl,
 $R4$ is hydroxy or methoxy,
15 L and the broken line indicates a single or a
double bond at the 22,23-position, provided that $R2$
is hydroxy only when the broken line indicates a
single bond.
 P The term "loweralkyl" when used in the
20 instant application is intended to represent those
alkyl groups either straight or branched chain which
have from 1⁵ to 5 carbon atoms. Examples of such alkyl
groups are methyl, ethyl, propyl, iso-propyl, butyl,
sec-butyl, pentyl, and the like.
37 25 P The term "loweralkanoyl" is intended to
include those alkanoyl groups containing from one to
five carbon atoms in either a straight or branched
chain. Examples of such alkanoyl groups are formyl,
acetyl, propenyl, butyryl, valeryl, and the like.
37 30 P The term "halogen" is intended to include
those halogen atoms fluorine, chlorine, bromine and
iodine.

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P One aspect of the preferred compounds of this invention is realized in the above structural formula when R_1 is

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PS 90 50 and R_1 is = O or NR_5R_6 and R_5 and R_6 are independently hydrogen, methyl or acetyl.

Further, examples of preferred compounds of the instant invention are:

- 15 P₀ 41 4"-keto avermectin Bla;
 L 4"-keto avermectin Blb;
 L 4"-keto-22,23-dihydro avermectin Bla;
 41 4"-keto-22,23-dihydro avermectin Blb;
 20 (41) 4"-deoxy-4"-amino avermectin Bla;
 (41) 4"-deoxy-4"-amino avermectin B2b;
 (41) 4"-deoxy-4"-amino-22,23-dihydro
 avermectin Bla;
 P₀ (41) 4"-deoxy-4"-amino-22,23-dihydro
 avermectin Blb;
 25 P₀ (41) 4"-deoxy-4"-acetyl amino avermectin Bla;
 L (41) 4"-deoxy-4"-acetyl amino avermectin Blb;
 L (41) 4"-deoxy-4"-acetyl amino-22,23-dihydro
 avermectin Bla;
 P₀ (41) 4"-deoxy-4"-acetyl amino-22,23-dihydro
 avermectin Blb;
 30 P₀ (41) 4"-deoxy-4"-dimethyl amino avermectin Bla;
 L (41) 4"-deoxy-4"-dimethyl amino avermectin Blb;

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- P₀ (41) 4"-deoxy-4"-dimethylamino-22,23-dihydro
 avermectin Bla;
 P₀ (41) 4"-deoxy-4"-dimethylamino-22,23-dihydro
 avermectin Blb;
 5 P₀ (41) 4"-deoxy-4"-p-chloro benzenesulfonylamino-
 22,23-dihydro avermectin Bla;
 P₀ (41) 4"-deoxy-4"-p-chloro benzenesulfonylamino-
 22,23-dihydro avermectin Blb;
 10 P₀ (41) 4"-deoxy-4"-(2-methylbenzenesulfonylamino)-
 avermectin Bla;
 P₀ (41) 4"-deoxy-4"-(2-methylbenzenesulfonylamino)-
 avermectin Blb.

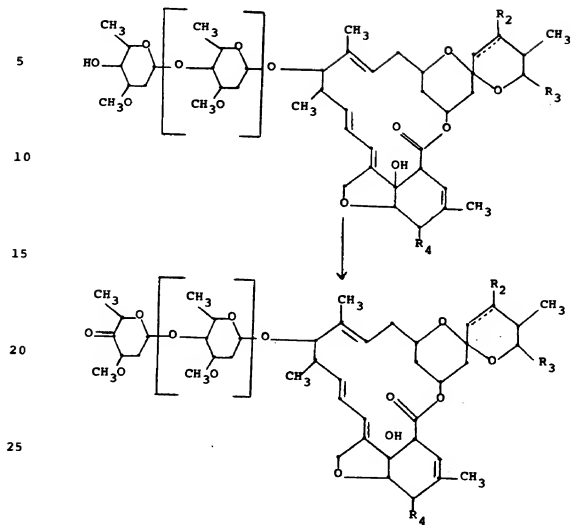
P 37 37 The "b" compounds, those with a
 25-iso-propyl group, are very difficult to separate
 37 15 37 from the corresponding "a" compound with a
 25-sec-butyl group and as such the compounds are
 37 37 generally isolated as mixtures of the two compounds.
 20 Thus references in the instant application to "a"
 37 37 compounds such as Bla, Ala, and the like, are
 construed to actually contain a certain proportion of
 the corresponding "b" compound. Alternatively, this
 representation of a mixture is sometimes done by
 referring to the B1 or B2 compounds or by separating
 the "a" compound from the "b" compound by a slash (/)
 37 37 such as Bla/Blb, B2a/B2b and the like.

P The compounds of the instant invention are
 prepared using the procedures exemplified in the
 following reaction scheme:

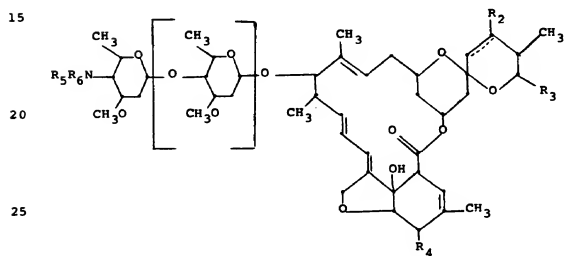
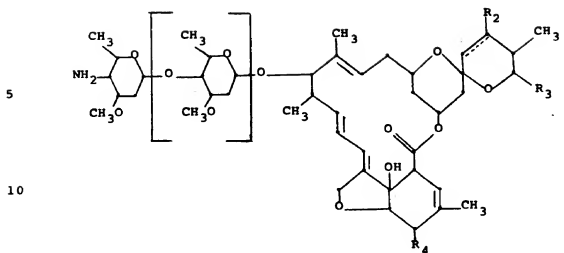
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PC wherein R_2 , R_3 and R_4 are as previously defined and the brackets indicate that the saccharide group contained therein may be either present or absent in the molecule.

5 P In the first step of the foregoing reaction scheme, the avermectin starting materials which may be either the naturally occurring products, the 22,23-dihydro derivatives thereof or the
10 monosaccharide derivative thereof, are oxidized at
41 the 4"-position to the corresponding keto compound. During the procedure the presence of any hydroxy groups at the 5 and 23-positions will require that such hydroxy groups be protected in order that they
15 too are not oxidized. The 7-hydroxy group is very non-reactive and inert and need not be protected. The procedure used to prepare the protected intermediates are described below. The oxidation reaction is carried out in an inert solvent such as
20 methylene chloride using oxalyl chloride or trifluoroacetic anhydride in dimethylsulfoxide as the oxidizing agent. Additionally N-chlorosuccinimide in dimethylsulfide may be employed. The reaction proceeds by dissolving the oxalyl chloride or
25 trifluoroacetic anhydride and dimethylsulfoxide (or other oxidizing reagents) in methylene chloride and
31 20 cooling to from -50 to -80°C and adding dropwise a methylene chloride solution of the avermectin compound to be oxidized. The addition is carried out
30 over a period of from 15 minutes to 1 hour and then triethylamine is added dropwise over a period of from 1 to 15 minutes. The reaction mixture is then allowed to warm to room temperature over a

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178 41 period of from 1/2 to 1 hour. The 4"-keto compound is isolated using techniques known to those skilled in the art.

41 In the next step, the 4"-keto compound is aminated to prepare the unsubstituted amino compound (R₅=R₆=hydrogen). The reaction is carried out in an inert solvent such as methanol at from -25 to +10°C using ammonium salts and sodium cyanoborohydride as the aminating and reducing reagents.

30 21 10 The reaction is complete in from 15 minutes to 2 hours and the product 4"-deoxy-4"-amino compound is isolated using techniques known to those skilled in the art. Suitable ammonium salts are the acetate, propionate, benzoate and the like. The acetate is preferred.

15 As a variation to the foregoing amination reaction, methyl ammonium salts could be used in place of the ammonium salts to prepare the monomethyl substituted compound directly. The same reagents, salts and reaction conditions as described above can be used for such a reaction.

25 The substitution reaction wherein the substituent is an acyl function is carried out using an acylating reagent in the presence of a base in an inert solvent. The preferred acylating reagents are loweralkanoyl anhydrides, loweralkanoyl halides, substituted benzene sulfonyl chlorides, lower alkyl sulfonyl chlorides, and the like. The reaction is carried out in an inert solvent such as methylene chloride in the presence of a non-reactive base such as pyridine or triethylamine in order to neutralize the acid produced during the course of the reaction.

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- 31 20 The reaction temperature is from -10 to 25°C and the reaction is complete in from 5 minutes to 1 hour. The product is isolated using known techniques.
- (41) 5 The reaction for the preparation of the 4"-deoxy-4"-dialkylamino compounds is carried out using the alkylating reaction conditions of formaldehyde and a reducing agent such as sodium borohydride, in methanol. The reaction is carried out in aqueous medium using excess aqueous
- 10 formaldehyde along with the presence of a small amount of acid such as acetic acid to facilitate the
- 31 30 20 reaction. The reaction is carried out at from -10 to +10°C with the solution of the avermectin compound in methanol added dropwise over a period of from 30 to
- 15 60 minutes to the alkylating reagent mixture and the product is isolated using known techniques.

CL PREPARATION OF STARTING MATERIALS

- P The ultimate starting materials for the
- 20 compounds of this invention are the avermectin fermentation products defined above. Thus it is apparent that additional reactions are required to prepare the instant compounds. Specifically,
- 25 reactions are carried out at the 5, 13, 22, and 23-positions. It is generally preferred to prepare whatever substituents are required at these positions
- 41 before the oxidation at the 4"-hydroxy and subsequent
- L substitution on the thus produced 4"-keto. Such a procedure generally avoids undesirable side
- 30 reactions. This technique is not required, however, and if desired other sequences may be used. In addition, during the oxidation and substitution

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reaction described above, it is necessary to protect the hydroxy groups at the 5- and 23-positions to avoid oxidation or substitution at such positions. With these positions protected the reactions may be

5 4) carried out at the 4"-position without affecting the remainder of the molecule. Subsequent to any of the above described reactions the protecting group may be removed and the unprotected product isolated. The protecting group employed is ideally one which may be

10 readily synthesized, will not be affected by the 4) reactions at the 4"-position and may be readily removed without affecting any other functions of the molecule. One preferred type of protecting group for the avermectin type of molecule is the

15 tri-substituted silyl group, preferably the trialkyl silyl group. One especially preferred example, is the t-butyl dimethylsilyl group. The reaction preparing the protected compound is carried out by reacting the hydroxy compound with the appropriately

20 substituted silylhalide, preferably the silylchloride in an aprotic polar solvent such as dimethylformamide. Imidazole is added as a catalyst. The reaction is complete in from 1 to 24 hours and at from 0 to 25°C. For the 5-position

178 25 hydroxy group the reaction is complete in from 1/2 to 20 3 hours at from 0°C to room temperature. This reaction is selective to the 5 position under the conditions above described and very little, if any, silylation is observed at other hydroxy substituted

30 positions. If it is desired to protect the 4) 23-hydroxy group a 4", 5,23-tri(phenoxyacetyl) derivative can be prepared. Basic hydrolysis will

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41 leave the highly hindered 23-O-substituent but
hydrolyze the 5- and 4"-O-phenoxy acetyl groups. The
5-position is then protected as described above,
selectively with t-butyldimethylsilyl.

5 The silyl group may be removed after the
other contemplated reactions may be carried out. The
silyl group or groups are removed by stirring the
silyl compound in methanol catalyzed by a catalytic
amount of an acid preferably a sulfonic acid such as
p-toluene sulfonic acid. The reaction is complete in
10 about 1 to 12 hours at from 0° to 50°C.

20 P Another of the starting materials used in
the foregoing reaction scheme are those in which the
22,23 double bond of the "1" type compounds has been
37 38 reduced to a single bond. As is readily apparent
15 from an analysis of the structure of avermectin
starting materials there are 5 unsaturations in the
1-series of compounds. Thus in the one series of
compounds it is necessary to reduce the 22,23 double
20 bond while not affecting the remaining four
unsaturations or any other functional group present
on the molecule in order to selectively prepare the
22,23 dihydro avermectins. It is necessary to select
a specific catalyst for the hydrogenation, one that
25 will selectively hydrogenate the least hindered from
among a series of unsaturations. The preferred
catalyst for such a selective hydrogenation procedure
is one having the formula: PS

PS 30 wherein $Tl \cdot [(R_7)_3P]_3RhY$ PS
2 R_7 is loweralkyl, phenyl or loweralkyl
substituted phenyl and Y is halogen. The reduction
procedure is completely described in U.S. Patent No.
4,199,569.

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P The other starting materials which are used in the above reaction scheme involve the preparation of the mono-saccharide compound. That is those compounds wherein one of the α -1-oleandrosyl groups have been removed. The removal of the terminal α -1-oleandrose leaves a hydroxy group at the 4'-position which is equally amenable to the reactions described in the foregoing reaction scheme. Of course in such a case the products prepared are 4'-keto and 4'-deoxy 4'-amino derivatives rather than the 4"-keto and 4"-deoxy 4"-amino derivatives. The processes which may be used to prepare the monosaccharide derivatives of the avermectin compounds are described in U.S. Patent No. 4,206,205. The reaction consists generally of treating the starting material disaccharide with acid in an aqueous organic solvent mixture. Water concentrations of from 0.1 to 20% by volume and acid concentrations of from about 0.01 to 0.1% will predominantly produce the monosaccharide product.

A further procedure for the preparation of the monosaccharide utilizes a 1% mineral acid solution in isopropanol at for 20-40°C preferably at room temperature for from 6 to 24 hours. Mineral acids such as sulfuric, hydrohalic, phosphoric and the like may be employed.

The novel compounds of this invention have significant parasiticidal activity as anthelmintics, ectoparasiticides, insecticides and acaricides, in human and animal health and in agriculture.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic

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problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris and Parascaris. Certain of these, such as Nematodirus, Cooperia and Oesophagostomum attack primarily the intestinal tract while others, such as Haemonchus and Ostertagia, are more prevalent in the stomach while still others such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels, subcutaneous and lymphatic tissue and the like. The parasitic infections known as helminthiasis lead to anemia, malnutrition, weakness, weight loss, severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host. The substituted avermectin compounds of this invention have unexpectedly high activity against these parasites, and in addition are also active against Dirofilaria in dogs, Nematospiroides, Syphacia, Aspiculuris in rodents, arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, in sheep

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Lucilia sp., biting insects and such migrating dipterous larvae as Hypoderma sp. cattle, Gastrophilus in horses, and Cuterebra sp. in rodents.

The instant compounds are also useful
5 against parasites which infect humans. The most common genera of parasites of the gastro-intestinal tract of man are Ancylostoma, Necator, Ascaris,
Strongyloides, Trichinella, Capillaria, Trichuris,
10 and Enterobius. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastrointestinal tract are the filarial worms such as Wuchereria, Brugia,
Onchocerca and Loa, Dracunculus and extra intestinal stages of the intestinal worms Strongyloides and
15 Trichinella. The compounds are also of value against arthropods parasitizing man, biting insects and other dipterous pests causing annoyance to man.

The compounds are also active against household pests such as the cockroach, Blatella sp.,
20 clothes moth, Tineola sp., carpet beetle, Attagenus sp., and the housefly Musca domestica.

The compounds are also useful against insect pests of stored grains such as Tribolium sp.,
Tenebrio sp. and of agricultural plants such as
25 spider mites, (Tetranychus sp.), aphids, (Acyrtosiphon sp.); against migratory orthopterans such as locusts and immature stages of insects living on plant tissue. The compounds are useful as a nematocide for the control of soil nematodes and
30 plant parasites such as Meloidogyne spp. which may be of importance in agriculture. The compounds are active against other plant pests such as the southern army worm and Mexican bean beetle larvae.

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These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient usually in water together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contains from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the avermectin derivatives in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

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When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the antiparasitic compounds of our invention may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cotton seed oil and the like. Other parenteral vehicles such as organic preparation using solketal, glycerol formal, and aqueous parenteral formulations are also used. The active avermectin compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

Although the antiparasitic agents of this invention find their primary use in the treatment and/or prevention of helminthiasis, they are also useful in the prevention and treatment of diseases caused by other parasites, for example, arthropod parasites such as ticks, lice, fleas, mites and other biting insects in domesticated animals and poultry. They are also effective in treatment of parasitic diseases that occur in other animals including humans. The optimum amount to be employed for best

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results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally good results are
5 obtained with our novel compounds by the oral administration of from about 0.001 to 10 mg per kg of animal body weight, such total dose being given at one time or in divided doses over a relatively short period of time such as 1¹/₅ days. With the preferred
10 compounds of the invention, excellent control of such parasites is obtained in animals by administering from about 0.025 to 0.5 mg per kg of body weight in a single dose. Repeat treatments are given as required to combat re-infections and are dependent upon the
15 species of parasite and the husbandry techniques being employed. The techniques for administering these materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are
20 administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant
25 one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

30 Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are

suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or dilutents suitable for such compositions

5 3 include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like. The active hydrogenated

10 avermectin compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from about 0.005 to 2.0% by weight of the active compound are particularly suitable as feed

15 premixes. Feed supplements, which are fed directly to the animal, contain from about 0.0002 to 0.3% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the

20 concentration of active compound desired for the treatment and control of parastic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular avermectin derivative

25 employed, the compounds of this invention are usually fed at concentrations of between 0.00001 to 0.002% in the feed in order to achieve the desired antiparasitic result.

The avermectin compounds of this invention

30 are also useful in combatting agricultural pests that inflict damage upon crops while they are growing or while in storage. The compounds are applied using

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known techniques as sprays, dusts, emulsions and the like, to the growing or stored crops to effect protection from such agricultural pests.

In using the compounds of this invention, the individual substituted avermectin components may be prepared and used in that form. Alternatively, mixtures of two or more of the individual avermectin components may be used, as well as mixtures of the parent avermectin compounds, other avermectin compounds or other active compounds not related to avermectin, with the compounds of this invention.

41 The 4"-amino and substituted amino compounds of the present invention are valuable antibiotics active against various Gram-positive and Gram-negative bacteria and accordingly find utility in human and veterinary medicine. Representative pathogens which are sensitive to the instant compounds include: Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Salmonella typhosa, Pseudomonas and Bacterium proteus. The antibacterials of the invention are not limited to utility as medicaments; they may be used in all manner of industry, for example: additives to animal feed, preservation of food, disinfectants, and in other industrial systems where control of bacterial growth is desired. For example, they may be employed in aqueous compositions in concentrations ranging from 0.1 to 100 parts of antibiotic per million parts of solution in order to destroy or inhibit the growth of harmful bacteria on medical and dental equipment and as bactericides in industrial applications, for example in waterbased paints and in the white water of paper mills to inhibit the growth of harmful bacteria.

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The products of this invention may be used in any of a variety of pharmaceutical preparations. They may be employed in capsule, powder form, in liquid solution, or in suspension. They may be administered by a variety of means; those of principal interest include: orally, topically or parenterally by injection (intravenously or intramuscularly).

Such tablets and capsules, designed for oral administration, may be in unit dosage form, and may contain conventional excipients, such as binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example, lactose, sugar, cornstarch, calcium phosphate, sorbitol, or glycerine; lubricants, for example, magnesium stearate, talc, polyethylene glycol, silica; disintegrants, for example, potato starch, acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of aqueous or oily suspensions, or solutions, or they may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, or carboxymethyl cellulose. Suppositories will contain conventional suppository bases, such as cocoa butter or other glycerides.

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Compositions for injection, the preferred route of delivery, may be prepared in unit dosage form in ampules, or in multidose containers. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatortory agents. Alternatively, the active ingredient may be in powder form for reconstitution, at the time of delivery, with a suitable vehicle, such as sterile water.

The compositions may also be prepared in suitable forms for absorption through the mucous membranes of the nose and throat or bronchial tissues and may conveniently take the form of liquid sprays or inhalants, lozenges, or throat paints. For medication of the eyes or ears, the preparation may be presented in liquid or semi-solid form. Topical applications may be formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints, or powders.

The dosage to be administered depends to a large extent upon the condition and size of the subject being treated as well as the route and frequency of administration. The parenteral route by injection being preferred for generalized infections. Such matters, however, are left to the routine discretion of the therapist according to principles of treatment well known in the antibiotic art. In general, a daily dosage consists of from about 0.1 to about 5 mg of active ingredient per kg. of body weight of the subject in one or more treatments per day. A preferred daily dosage for adult humans lies in the range of from about 0.1 to

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20 mg. of active ingredient per kg. of body weight. Another factor influencing the precise dosage regimen, apart from the nature of the infection and peculiar identity of the individual being treated, is the molecular weight of the chosen species of this invention.

The compositions for human delivery per unit dosage, whether liquid or solid, may contain from 0.1% to 99% of active material, the preferred range being from about 10-60%. The composition will generally contain from about 5 mg. to about 50 mg. of the active ingredient; however, in general, it is preferable to employ a dosage amount in the range of from about 5 mg to 100 mg. In parenteral administration, the unit dosage is usually the pure compound I in sterile water solution or in the form of a soluble powder intended for solution.

In the isolation of the avermectin compounds, which serve as starting materials for the instant proceses, from the fermentation broth, the various avermectin compounds will be found to have been prepared in unequal amounts. In particular an "a" series compound will be prepared in a higher proportion than the corresponding "b" series compound. The difference between the "a" series and "b" series is constant throughout the avermectin compounds and consists of a sec-butyl group and an iso-propyl group respectively at the 25 position. This difference, of course, does not interfere with any of the instant reactions. In particular it may not be necessary to separate the "b" components from the related "a" component. Separation of these

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37 28 closely related compounds is generally not practiced since the "b" compound is present only in a very small percent by weight, and the structural difference has negligible effect on the reaction processes and biological activities.

5 In particular it has been found that the starting materials for the compounds of this invention are very often prepared in a ratio of about 80% avermectin Bla or Ala and 20% avermectin Blb or Alb. Thus the preferred composition of this
10 37 38 invention is one which contains about 80% of the "a" component and 20% of the "b" component.

6 The following examples are provided in order that this invention might be more fully understood;
15 they are not to be construed as limitative of the invention.

The substituted avermectin derivatives prepared in the following examples are generally isolated as amorphous solids and not as crystalline
20 solids. They are thus characterized analytically using techniques such as mass spectrometry, nuclear magnetic resonance, and the like. Being amorphous, the compounds are not characterized by sharp melting points, however, the chromatographic and analytical
25 methods employed indicate that the compounds are pure.

In the following examples, the various starting materials therefor are avermectin compounds or derivatives of avermectin compounds. The avermectin compounds and the preparation and
30 isolation thereof from fermentation broths are described in United States Patent No. 4,310,519 issued 12 January 1982. The selective 22,23-dihydro

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derivatives of avermectin compounds are described in U.S. Patent ^{No.} 4,199,569 issued (22 April) 1980. The monosaccharide derivatives of avermectin compounds are described in U.S. Patent ^{No.} 4,206,205 issued 3 January 1980.

DE CL EXAMPLE 1

CL 5-O-t-butyl-dimethylsilyl-22,23-dihydro avermectin
10 Bla/Blb

P 3 g of 22,23-dihydro avermectin Bla/Blb in 30 ml of dry dimethylformamide was combined with 1.4 g of imidazole and stirred at room temperature until
15 all the materials had dissolved. Then 1.56 g of t-butyl-dimethylsilyl chloride was added and the reaction mixture stirred at room temperature for 70 minutes. The reaction mixture was diluted with 150 ml of ether, water was added and the layers were
20 separated. The aqueous layer was extracted twice more with ether and the combined ether layers washed four times with water and once with saturated sodium chloride solution. The ether layer was dried over magnesium sulfate and concentrated to dryness in
25 vacuo affording 4.2 g of a white foam. The foam is chromatographed on 135 g. of 70-230 mesh silica gel and eluted with 5% tetrahydrofuran in methylene
41 chloride. 1.15 G of 4"-5-di-O-t-butyl-dimethylsilyl-22,23-dihydro avermectin Bla/Blb and 2.6 g of
30 5-O-t-butyl dimethylsilyl-22,23-dihydro avermectin Bla/Blb were recovered as pure amorphous foams.

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CL

EXAMPLE 2

CL 41 5-O-t-butyl-dimethylsilyl-4"-keto-22,23-
dihydro-avmerectin Bla/Blb

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31 20 P In a dried flask purged with dry nitrogen
1082 was placed 97 μ l of oxalyl chloride and 1.5 ml of
methylene chloride. The reaction mixture was cooled
to -60°C , 1 ml of the methylene chloride solution
31 20 containing 160 μ l of dimethylsulfoxide was added over
a period of 3 minutes and the reaction mixture
21 20 stirred at -60°C for two minutes. 3 Ml of methylene
chloride containing 500 mg of 5-O t-butyl-
dimethylsilyl 22,23-dihydro avermectin Bla/Blb was
15 added dropwise over a period of 5 minutes and the
reaction mixture stirred at room temperature for 30
minutes. At the end of this period, 0.71 ml of
triethylamine was added dropwise and the reaction
31 26 mixture was stirred at -60°C for 5 minutes. The cold
20 bath was removed and the reaction mixture was allowed
to come to room temperature over a period of 45
minutes. 50 Ml of water was added and the reaction
mixture was extracted 3 times with 40 ml of ether.
The ether extracts were combined and washed 4 times
25 with 20 ml of water, dried over magnesium sulfate and
concentrated to dryness in vacuo affording 520 mg of
a yellow glass. The yellow glass was dissolved in
methylene chloride and placed on three 2,000 μ silica
gel preparative layer chromatography plates. The
30 plates were developed with 10% ethyl acetate in
methylenchloride and afforded 470 ml of yellow foam
which was characterized by its 300 MHz nuclear

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41 magnetic resonance spectrum as 5-O-t-butyl-dimethyl-
silyl-4"-keto-22,23-dihydro avermectin Bla/Blb.

CL

EXAMPLE 3

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CL

5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-amino-
22,23-dihydro avermectin Bla/Blb

- P Into a dried flask was placed 200 mg of 5-O-
10 41 t-butyl-dimethylsilyl-4"-keto-22,23-dihydro
-avermectin Bla/Blb 2 ml of methanol, 160 mg of
ammonium acetate and 12 mg of sodium cyano-
borohydride. The reaction mixture was stopped and
stirred at room temperature for 100 minutes. The
15 reaction mixture was added to a 10 ml solution of
saturated sodium carbonate diluted 1 to 1 with water
to afford a gummy precipitate. The precipitate was
extracted 4 times with 3 ml of ethyl acetate and the
ethyl acetate layers were combined and washed four
20 times with 1 ml of water. The organic layer was
dried over magnesium sulfate and evaporated to
dryness in a stream of nitrogen to afford 198 mg of
yellow opaque glass. The glass was dissolved in
82 methylene chloride and placed on three 1,000 μ silica
25 gel preparative layer chromatography plates and
eluted with 7% methanol in methylene chloride to
afford 19 mg of a yellow glass which was identified
by 300 MHz nuclear magnetic resonance as 5-O-
41 t-butyl-dimethylsilyl-4"-~~deoxy~~-4"-amino-22,23-
30 dihydro avermectin Bla/Blb and 64 mg of a yellow

30

glass which was identified by 300 MHz nuclear

41 magnetic resonance as 5-O-t-butyl-dimethylsilyl-4"-
deoxy-4"-epiamino-22,23-dihydro avermectin Blb/Blb.

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CL

EXAMPLE 4

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(41)

4"-deoxy-4"-amino-22,23-dihydro-avermectin Bla/Blb

(41)

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P 17 mg of 5-O t-butyl-dimethylsilyl
-4"deoxy-4"-amino-22,23-dihydro avermectin Bla/Blb
was dissolved in a few drops of methanol and cooled
in an ice-bath with stirring. 3 ml of 1% (weight to
volume), p-toluene sulfonic acid hydrate solution in
methanol was added and the reaction mixture stirred
15 for 55 minutes. The reaction mixture was added to
dilute sodium bicarbonate solution and extracted 3
times with 3 ml portions of ethyl acetate. The
organic layers were combined and washed 3 times with
1 ml of water and once with saturated sodium
20 chloride solution. The organic layer was dried over
magnesium sulfate and evaporated to dryness under a
stream of nitrogen affording 18 mg of a yellow
glass. The yellow glass was dissolved in methylene
chloride and placed on a 250 u silica gel preparative
25 layer chromatography plate and eluted with 8%
methanol in methylene chloride. The band with an Rf
of 0.20 afforded 8.5 mg of a white glass. Mass
spectrometry and 300 MHz nuclear magnetic resonance
identified it as 4"-deoxy-4"-amino-22,23-dihydro-
30 avermectin Bla/Blb.

(41)

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CL

EXAMPLE 5

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(41)

4"-deoxy-4"-epi-amino-22,23-dihydro avermectin Bla/Bl
b

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P

(41)

Following the procedure of Example 4 using
60 mg of 5-O-t-butyl-dimethylsilyl 4"-deoxy-4"
epi-amino-22,23-dihydro avermectin Bla/Blb 6.0 ml of
1% weight to volume p-toluene sulfonic acid hydrate

10 (41)

in methanol there was obtained 27.4 mg of 4"-deoxy-4"
epi-amino-22,23-dihydro avermectin Bla/Blb which
structure was confirmed by mass spectrometry and 300
MHz nuclear magnetic resonance.

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EXAMPLE 6

CL

(41)

5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-acetyl-amino
22,23-dihydro avermectin Bla/Blb

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P

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30 mg of 5-O-t-butyl-dimethylsilyl-4"
deoxy-4" amino-22,23-dihydro avermectin Bla/Blb was
combined with 0.5 ml of methylene chloride and cooled
in an ice-bath. 6 Drops of pyridine was added
followed by 2 Drops of acetic anhydride. The
reaction mixture was stirred at 0°C for 30 minutes.
Water and ether were added, the layers were separated
and the aqueous layer extracted again with ether.

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The organic layers were combined and washed four
times with 1 ml portions of water, dried over
magnesium sulfate and evaporated to dryness under a
stream of nitrogen to afford 35 mg of a yellow
glass. The yellow glass was dissolved in methylene

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82 chloride and placed on a 500 u silica gel preparative layer chromatography plate and eluted with a 1 to 1 mixture of ethyl acetate in methylene chloride to afford 14.6 mg of off-white foam. 300 MHz nuclear magnetic resonance characterized the material as 5-O-⁽⁴¹⁾ t-butyl-dimethylsilyl-4"-deoxy-4"-acetyl-amino-22,23-dihydro-avermectin Bla/B1b.

CL

EXAMPLE 7

CL ¹⁰ ⁽⁴¹⁾ 4"-deoxy-4"-acetyl-amino-22,23-dihydro avermectin Bla/B1b

⁽⁴¹⁾ P Following the procedure of Example 4, 4"-acetyl-amino-4"-deoxy-5-O-t-butyl-dimethylsilyl-22,23-dihydroavermectin Bla/B1b was treated with a 1% solution of p-toluenesulfonic acid monohydrate in methanol (w/v) to give after isolation and ⁽⁴¹⁾ purification 4"-acetyl-amino-4"-deoxy-22,23-dihydro-avermectin Bla/B1b.

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CL

EXAMPLE 8

CL ⁽⁴¹⁾ 5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-epi-acetyl-amino-22,23-dihydro-avermectin Bla/B1b

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⁽⁴¹⁾ P Following the procedure of Example 6 wherein 5 mg of 5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-epi-amino-22,23-dihydro avermectin Bla/B1b was employed, there was obtained 5-O-t-butyl-dimethylsilyl 4"-deoxy-4"-epi-acetyl-amino-22,23-dihydro ⁽⁴¹⁾ avermectin Bla/B1b.

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CL

EXAMPLE 9

CL (41)

4"-deoxy-4"-epi-acetyl-amino-22,23-
dihydro-avermectin Bla/Blb

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P

Following the procedure of Example 4 in
41 which 35 mg of 5-O-t-butyl-dimethylsilyl-4"⊖
L deoxy-4"-epi-acetyl-amino-22,23-dihydro avermectin
Bla/Blb was employed with 4 ml of 1% weight to volume
p-toluene sulfonic acid hydrate in methanol. There
10 (41) was obtained 19.4 mg of 4"-deoxy-4"-epi-acetyl-amino-
22,23-dihydro-avermectin Bla/Blb.

CL

EXAMPLE 10

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CL

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4"-keto-22,23-dihydro-avermectin Bla/Blb

P

25 mg of 5-O-t-butyl-dimethylsilyl-4"⊖
22,23-dihydro-avermectin Bla/Blb in 3 mg of 1%
20 weight to volume p-toluene sulfonic acid hydrate in
methanol was stirred at room temperature for 20
minutes. 20 Ml of water was added and the reaction
mixture extracted three times with 15 ml portions of
ether. The ether layers were combined and washed
25 three times with 3 ml portions of water dried over
magnesium sulfate and evaporated to dryness in vacuo
to afford 15 mg of a colorless glass. The glass was
dissolved in methylene chloride and placed on a 1000
μ silica gel preparative layer chromatography plate
30 and eluted with 5% methanol in methylene chloride to
afford 14.5 mg of an off-white glass characterized by

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41 mass spectrometry and 300 MHz nuclear magnetic resonance as 4"-keto 22,23-dihydro-avermectin Bla/Blb.

CL EXAMPLE 11

CL⁵ (41) 5-O-t-butyl dimethylsilyl-4"-deoxy-4"-epi-
N,N-dimethylamino-22,23-dihydro avermectin Bla/Blb

P 50 mg of 5-O-t-butyl-dimethylsilyl-4"-
10 41 deoxy-4"-epi-amino-22,23-dihydro-avermectin Bla/Blb
was dissolved in 0.7 ml of methanol and 0.4 ml of
acetic acid added followed by 0.5 ml of 37% aqueous
formaldehyde solution. The reaction mixture was
15 stirred at room temperature for 30 minutes, cooled in
an ice bath and 130 mg of sodium borohydride was
added in 5-10 mg portions with 5-10 minutes
separating each portion. Vigorous foaming
accompanied each addition. A saturated sodium
bicarbonate solution was added in small portions to
20 the reaction mixture to neutrality followed by
extraction with ether. The aqueous layer was
extracted twice more with 20 ml portions of ether.
The ether layers were combined and washed four times
with 4 ml portions of water. The combined organic
25 layers were dried over magnesium sulfate and
evaporated to dryness in vacuo affording 40 ml of a
slightly yellow glass. The glass was dissolved in
82 methylene chloride and placed on a 1000 u silica gel
preparative layer chromatography plate and developed
30 with ethyl acetate to afford 30.5 mg of yellow glass
identified by mass spectrometry as 5-O-t-butyl-
(41) dimethylsilyl-4"-deoxy-4"-epi-N,N-dimethylamino-
22,23-dihydro avermectin Bla/Blb.

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EXAMPLE 12

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(41)

4"-deoxy-4"-epi-N,N-dimethylamino-
22,23-dihydro-avermectin Bla/B1b

5 P

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29 Mg of 5-O-t-butyl-dimethylsilyl-4"-
deoxy-4"-epi-N,N-dimethylamino-22,23-dihydro
avermectin Bla/B1b was treated with 3 ml of 1% weight
to volume p-toluene sulfonic acid hydrate in methanol
following the procedure of Example 4. There was
afforded 15.8 mg of a white glass which was
identified by mass spectrometry and 300 MHz nuclear
magnetic resonance as 4"-deoxy-4"-epi-N,N-dimethyl-
amino-22,23-dihydro-avermectin Bla/B1b.

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CL

EXAMPLE 13

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(41)

5-O-t-Butyldimethylsilyl-4"-deoxy-4"- (4-chloro-
phenylsulfonylamino)-22,23-dihydroavermectin-Bla/B1b

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P

A solution of 100 mg of 5-O-t-butyldimethyl-
silyl-4"-deoxy-4"-amino-22,23-dihydroavermectin
Bla/B1b is dissolved in 2 ml of methylenechloride and
treated with 70 mg of triethylamine and 45 mg of
4-chlorobenzenesulfonylchloride at room temperature
for 16 hrs. The usual work-up gives 5-O-t-butyl-
dimethylsilyl-4"-deoxy-4"- (4-chlorophenylsulfonyl-
amino)-22,23-dihydroavermectin-Bla/B1b in pure form.

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EXAMPLE 14

CL (41) 4"-Deoxy-4"-(4-chlorophenylsulfonylamino)-
22,23-dihydroavermectin-Bla/Blb

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P

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100 Mg of 5-O-t-butyldimethylsilyl-4"-deoxy-
4"-(4-chlorophenylsulfonylamino)-22,23-dihydro-
avermectin-Bla/Blb is treated according to Example 4
with a solution of 1% of p-toluenesulfonic acid
monohydrate in methanol for 30 min at room
temperature affording
(41) 4"-deoxy-4"-(4-chlorophenylsulfonylamino)-22,23-
dihydroavermectin-Bla/Blb in pure form.

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CL

EXAMPLE 15

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41

4"-Keto-5-O-t-butyldimethylsilylvermectin-Bla/Blb

P

20 41 If avermectin Bla/Blb is reacted according
to the procedures of Examples 1 and 2 4"-keto-5-O-t-
butyldimethylsilylvermectin-Bla/Blb is obtained.

CL

EXAMPLE 16

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4"-Keto-avermectin-Bla/Blb

P

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If the product of Example 15 is reacted
according to the procedure of Example 10, 4"-keto-
avermectin-Bla/Blb is obtained.

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EXAMPLE 17

CL (41) 4"-Amino-4"-deoxyavermectin Bla/Blb

5

P If the product of Example 15 is reacted according to the procedures of Examples 3 and 4 4"-amino-4"-deoxyavermectin Bla/Blb is obtained.

CL EXAMPLE 18

10 CL (41) 4"-Acetylamino-4"-deoxavermectin Bla/Blb

15

P If the product of Example 15 is reacted according to the procedures of Examples 3, 6 and 7 4"-acetylamino-4"-deoxavermectin Bla/Blb is obtained.

CL EXAMPLE 19

CL 40 4'-Keto-5-O-t-butyldimethylsilyl-22,23-dihydro-
avermectin Bla/Blb monosaccharide

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P If 22,23-dihydroavermectin Bla/Blb-mono-saccharide is reacted according to the procedures of Examples 1 and 2 4'-keto-5-O-t-butyldimethylsilyl 22,23-dihydroavermectin Bla/Blb monosaccharide is obtained.

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CL
CL 40
EXAMPLE 20

4'-Keto-22,23-dihydroavermectin
Bla/Blb-monosaccharide

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P
40 If the product of Example 19 is reacted according to the procedures of Example 10 4'-keto-22,23-dihydroavermectin Bla/Blb-monosaccharide is obtained.

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CL
CL (40) EXAMPLE 21

4'-Amino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb
monosaccharide

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P
(40) If the product of Example 19 is reacted according to the procedures of Examples 3 and 4, 4'-amino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb-monosaccharide is obtained.

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CL
CL (40) EXAMPLE 22

4'-Acetylamino-4'-deoxy-22,23-dihydro-
avermectin-Bla/Blb-monosaccharide

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P
(40) If the product of Example 19 is reacted according to the procedures of Examples 3, 6 and 7, 4'-acetylamino-4'-deoxy-22,23-dihydroavermectin Bla/Blb-monosaccharide is obtained.

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